U.S. DEPARTMENT OF AGRICULTURE GRAIN INSPECTION, PACKERS AND STOCKYARDS ADMINISTRATION FEDERAL GRAIN INSPECTION SERVICE STOP 3630 WASHINGTON, DC 20250-3630 DON HANDBOOK CHAPTER 18 11-19-07

CHAPTER 18

ENVIROLOGIX QUICKTOX DON 2 PPM TEST KIT

Section Number	ction Number Section	
18.1	GENERAL INFORMATION	18-1
18.2	PREPARATION OF EXTRACTION SOLUTION	18-1
18.3	EXTRACTION PROCEDURES	18-1
18.4	TEST PROCEDURES	18-1
18.5	INTERPRETING TEST RESULTS	18-3
18.6	REPORTING AND CERTIFYING TEST RESULTS	18-4
18.7	CLEANING LABWARE	18-4
18.8	WASTE DISPOSAL	18-4
18.9	EQUIPMENT AND SUPPLIES	18-4
18 10	STORAGE CONDITIONS	18-5

18.1 GENERAL INFORMATION

The QuickTox DON 2 ppm test kit is approved for wheat, and corn uses lateral flow test strip technology to provide qualitative results at a threshold of 2 ppm. Test samples are considered negative when the result is less than 2 ppm (< 2 ppm). Test samples are considered positive when the result is equal to or greater than 2 ppm (≥ 2 ppm).

18.2 PREPARATION OF EXTRACTION SOLUTION

The extraction solution used in the QuickTox DON 2 ppm test method (part number AS 104 BG) does not necessitate the use of a separate FGIS approved laboratory space. FGIS personnel may perform the testing in an FGIS approved laboratory space (i.e., table-top in an inspection lab) upon approval of the field office manager. FGIS employees must comply with all applicable safety and sanitation requirements as listed in the handbook to ensure a safe and efficient work environment.

18.3 EXTRACTION PROCEDURES

- a. Thoroughly mix ground sample and weigh a 50 gram portion.
- b. Place the ground 50 gram portion into an extraction mixing jar.
- c. Add 250 ml of **tap water** at room temperature.
- d. Cover the extraction jar and shake (by hand or mechanically) for 3 minutes. Ensure that entire sample appears completely wet and thoroughly mixed.
- e. Allow the sample to settle until fine particles are mostly settled (1 to 3 minutes). The top layer containing the DON residues will be used for testing.

18.4 TEST PROCEDURES

- a. Reaction Vial.
 - (1) Using the disposable transfer pipette included in the test kit, place 1 ml sample diluent (at room temperature) into each reaction vial.

NOTE: To ensure correct volumes are used to prepare each test sample, disposable transfer pipettes are included with each test kit.

(a) Squeeze the bulb tightly and insert the tip in the sample diluent, release pressure to draw liquid up past the 1 ml mark.

- (b) Squeeze carefully to expel excess diluent back into the bottle so that the liquid left in the pipette is at the 1 ml mark.
- (c) Expel 1 ml of diluent into the reaction vial.
- (2) Using a new pipette tip or new disposable transfer pipette for each reaction vial, transfer 50 µl from the top (tan) layer of the extract into the reaction vial containing sample diluent.
- (3) Mix sample diluent and sample extraction solution by stirring with the tip of the pipette.
- (4) After diluting the sample the final volume in the reaction vial should be $1050 \mu l$.
- (5) Test sample extract is now diluted and ready for test analysis

b. Test Strips.

- (1) Allow refrigerated canisters to reach room temperature before opening. Remove the QuickTox strips to be used then immediately reseal the canister. Avoid bending the strips. Use care not to dislodge the arrow tape on the end of the strip.
- (2) Place the strip into the reaction vial containing the diluted sample extract, and start timed reaction period (5 minutes). The arrow tape on the end of the strip should point into the reaction vial.
- (3) The sample extract will travel up the strip. Reaction vials can stand on their own, or may be inserted into the cardboard racks provided.
- (4) Allow the strip to develop for 5 minutes before making final assay interpretations. Strips should be read **promptly at 5 minutes while wet.**
- (5) If the strips are to be retained, cut off and discard the bottom section of the strip covered by the arrow tape. Discard all used reaction vials.
- (6) Negative Result.

A sample containing DON residue less than 2 ppm; the middle test line will be darker than the bottom 2.0 Reference Line.

(7) Positive Result.

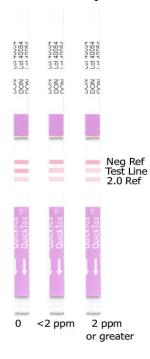
A sample containing DON residue equal to or greater than 2 ppm; the middle test line will be **equal to or lighter than the bottom 2.0 Reference Line.**

18.5 INTERPRETING TEST RESULTS

The QuickTox Strip for DON strip has three lines. The top line is a Negative Reference (NR) that develops a signal similar to a Negative DON sample. The bottom line is a 2 ppm Reference Line (2R) that develops a signal similar to a 2 ppm DON sample. The middle line is the Test Line and is compared to the bottom 2R Line. Results are invalid if either Reference Line fails to develop.

If the middle Test Line color intensity is: **Darker than the (Bottom) 2R Line** the sample is considered as negative "Less than 2 ppm DON", < 2 ppm.

If the middle Test Line color intensity is: **Equal to or lighter than the (Bottom) 2R Line** the sample is considered as positive "2 ppm or more DON", \geq 2 ppm



18.6 REPORTING AND CERTIFYING TEST RESULTS

a. Report results on the pan ticket and certify as being less than 2 ppm (< 2 ppm), or as equal to or greater than 2 ppm (≥ 2 ppm).

NOTE: Under the CuSum Loading Plan samples reported as 2 ppm or greater will be interpreted as exceeding the 2 ppm threshold and reported accordingly.

b. Refer to the DON Handbook Chapter 4 for detailed certification procedures when using this test kit.

18.7 CLEANING LABWARE

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

18.8 WASTE DISPOSAL

After the test has been completed, the remaining sample extracts and sample solutions may be poured down the drain. Discard solid material in the trash for routine disposal.

18.9 EQUIPMENT AND SUPPLIES

- a. <u>Materials Supplied in Test Kits.</u>
 - (1) 50 QuickTox strips packed in a moisture-resistant container.
 - (2) 50 transfer pipettes marked for 1 ml.
 - (3) 50 reaction vials.
 - (4) Sample diluent.

b. Materials Required but not Provided:

- (1) Timer (5 minute capacity).
- (2) Graduated cylinders.
- (3) Disposable transfer pipettes to dispense 50 µl.
- (4) Disposable plastic cups with tops.

- (5) Fixed volume pipette 100 μl.
- (6) Pipette tips 100 μl.
- (7) Balance.
- (8) Sample grinder.
- (9) Sample extraction jars.
- (10) **Tap water**.

18.10 STORAGE CONDITIONS

a. <u>Storage Conditions.</u>

Test kits should be refrigerated between 36°- 48°F.

- b. <u>Precautions.</u>
 - (1) Do not use the test kits beyond the noted expiration date.
 - (2) Prolonged exposure to high temperatures may adversely affect the test results.
 - (3) Do not open the desiccated canister until ready to use the strips.